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EFFECT OF FARMYARD MANURE AND MINERAL FERTILIZERS ON COLORATION, GROWTH, AND BIOMASS PRODUCTION OF *AZOLLA PINNATA*

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ABSTRACT

An experiment was conducted in ponds to determine the effect of farmyard manure and mineral fertilizers on the coloration, growth, and biomass production of *Azolla pinnata*. Dark green color was observed in the treatment supplied with farmyard manure and brilliant green color in the treatments supplied with phosphorus, and phosphorus plus nitrogen. Plants without any fertilizer were light green in color. The number of plants, and fresh and dry weight of *A. pinnata* were significantly higher ($P \leq 0.05$) in farmyard manure than other treatments. Addition of phosphorus, and phosphorus plus nitrogen also significantly (≤ 0.05) increased the number of plants, and fresh and dry weights of *A. pinnata* as compared to the control treatment. Results suggest that farmyard manure is more effective and cheaper than phosphorus, or phosphorus plus nitrogen for propagation and multiplication of *A. pinnata*.

KEY WORDS: *Azolla*, Azollaceae, fertilizer, ecology

INTRODUCTION

Azolla is a free floating aquatic fern belonging to the cryptogamic family Azollaceae. It forms symbiosis with the nitrogen-fixing cyanobacterium *Anabaena azollae* (Watanabe 1982). The importance of *Azolla* as an organic input in rice cultivation is extensively reported (Yanni 1991; Kumarasinghe & Eskew 1993; Yanni *et al.* 1994; Kundu & Ladha 1995). The genus is widely distributed throughout temperate and tropical regions with *A. pinnata* as the most prevalent species in Asia (Lumpkin 1987; Liu & Zheng 1992; Kundu & Ladha 1995).

Azolla varies in color under different environmental conditions. Growth of *Azolla* is also greatly affected by physico-chemical factors (Lumpkin 1987; Siddiqi *et al.* 1987; Bonetto & Carcano 1995). The *Azolla-Anabaena* symbiosis has been observed both in the presence of combined nitrogen and in a nitrogen free medium (Lumpkin 1987; Watanabe 1982). But the optimum growth of *Azolla* requires fertilization with phosphorus and sometimes with nitrogen (Watanabe *et al.* 1980; Aziz & Watanabe 1983; Watanabe & Ramirez 1990; Yanni *et al.* 1994). However, use of chemical fertilizers for *Azolla* culture is limited due to economic reasons and environmental restrictions (Kundu & Ladha 1995).

Azolla is found naturally in ditches, ponds and roadside streams in central Punjab, Pakistan. It is gaining importance in rice growing areas due to its nitrogen-fixing ability and green manuring property (Kumarasinghe & Eskew 1993; Kundu & Ladha 1995). The *Azolla-Anabaena* symbiosis can produce 1 ton of green manure per hectare per day containing 3 kg of fixed nitrogen which is equivalent to 15 kg of ammonium sulphate of 7 kg of urea (El-Bassel *et al.* 1994). The benefit of *Azolla* incorporation on the yield of rice has been well demonstrated. *Azolla* has been used as green manure for rice in China, Philippines, and northern Vietnam (Watanabe *et al.* 1989). The use of *Azolla* as green manure in wetland rice was summarized by Watanabe (1987) from internationally coordinated work by the International Network on Soil Fertility and Fertilizer Evaluation for Rice (INSFFER). Watanabe (1987) mentioned that incorporation of one crop of *Azolla* gave an increased yield equivalent to that given by 30 kg urea N per hectare. Siddiqi *et al.* (1985) and Bonetto & Carcano (1995) reported 16-19% grain yield increase over control when *Azolla* was incorporated into rice fields. The use of *Azolla* by farmers is limited by a number of constraints. The most important constraint faced in the use of *Azolla* is the large size of inoculum needed for a limited field area, especially with the difficulties of preservation and transportation (Kulasooriya 1991). Besides, *Azolla* must be produced and distributed fresh among rice farmers just before use. Attempts have been made to identify factors affecting growth and nitrogen-fixing ability of *Azolla-Anabaena* and to develop conditions suitable for its propagation, transportation and utilization in rice (Siddiqi *et al.* 1987; Kumarasinghe & Eskew 1993; Kulasooriya *et al.* 1994; Yanni *et al.* 1994). The current studies were designed to determine the effect of farmyard manure and mineral fertilizers on the coloration, growth, and biomass production of *Azolla*.

MATERIALS AND METHODS

The experiment was conducted in ponds constructed at the National Agricultural Research Center, Islamabad (33° 42′ N, 73° 7′ E, elevation approximately 518 m). *Azolla pinnata* was obtained from the Rice Research Institute, Kala Shah Kaku, Punjab, Pakistan. *Azolla* plants were cultured in ponds measuring 3 × 3 × 1 m filled with irrigation water from a nearby well. The bottom and side walls of all ponds were lined with polyethylene sheets. There were four treatments replicated four times in a completely randomized design. T-1 was control without addition of any fertilizer. T-2 received farmyard manure (FYM) at the rate of 10 ton/ha fresh weight. Farmyard

manure used in this experiment contained buffalo and cow dung, liquid animal excreta, and organic matter including rice and wheat straw. Farmyard manure contained 1.24% nitrogen, 0.75% phosphorus, and 1.07% potassium on a dry weight basis. T-3 was supplied with phosphorus as single super phosphate (SSP) at the rate of 5 kg/ha. T-4 was ameliorated with 5 kg/ha of phosphorus from single super phosphate plus one kg/ha nitrogen from urea. One kg (fresh weight) of healthy *Azolla* plants was added to each pond during late March. Population was recorded after two and five months in each pond. Sampling was done with a wooden quadrat measuring 50 × 50 cm taking five samples from each pond. Change in the color of *Azolla* was observed visually and population was recorded by counting the total number of plants per quadrat at each sampling. Biomass production per quadrat was expressed both as fresh and dry weight. Dry weight was determined by drying the plants in an oven at 60° C for 48 hours. Results were analyzed statistically by the method of Steele & Torrie (1980). Means were compared with the LSD multiple comparison test at $P \leq 0.05$.

RESULTS AND DISCUSSION

Azolla pinnata changed color from green to red within two weeks after application of treatments and remained red until the first week of April when the plants were four weeks old. With the increase in average day and night temperature from 20°/12° C during March to 26°/15° C during April, the color began to change to green. The average humidity was 85% during March and 61% during April. *Azolla* in all the four treatments was green in May, when average day/night temperature was 29°/20° C and average humidity was 71%. The color was again red in June/July when average day/night temperature was 37°/22° C and 36°/25° C, and average humidity was 54% and 70%, respectively.

The color of *Azolla pinnata* was light green in the control treatment but it was dark green when treated with farmyard manure. *Azolla* plants in the phosphorus treatment, and in phosphorus plus nitrogen treatment were brilliant green. Similar observations were made by Watanabe *et al.* (1980, 1989), Siddiqi *et al.* (1985) and Watanabe & Ramirez (1990) who reported that deficiency of phosphorus activates the production of anthocyanin which causes the appearance of red color in *Azolla*.

The research on *Azolla* culture points out several constraints to its universal use (Kulasooriya 1991; Kulasooriya *et al.* 1994; Bonetto & Carcano 1995). Among them, excessive light and temperature have been mentioned (Chung 1987; Fiore & Gutbrod 1987; Watanabe *et al.* 1989). It has been reported that red coloration in *Azolla* due to anthocyanin formation does not affect its growth and nitrogen-fixing ability (Chung 1987; Lumpkin 1987). The change in the color of *Azolla* at the beginning of the experiment may be due to sudden change in environment including low temperature which produced physiological stress in the plants. The average temperature increased from 26°/15° C during April to 29°/20° C during May, and humidity also changed from 61% during April to 71% during May providing optimum conditions for the

growth of *Azolla*. *Azolla* showed green coloration and grew vigorously during this period. The humidity for the normal growth of *Azolla* is above 60% but optimal values range from 85% to 90% (Watanabe 1982). Average temperature increased to 37° C in June reducing the humidity by as much as 54%. Plants developed red color indicating stress conditions. The next change in color was observed during September/October when average humidity was 50% and average day/night temperature was 33°/21° C and 25°/15° C, respectively. The color again changed to red with a drop in temperature below 22°/10° C during the later months.

The number of plants and biomass production of *Azolla pinnata* in various treatments is presented in Table 1. The number, fresh and dry weight of *Azolla* plants two months after treatment were significantly higher ($P \leq 0.05$) with farmyard manure than with other treatments. Addition of phosphorus, and phosphorus plus nitrogen also significantly ($P \leq 0.05$) increased the growth and biomass of *A. pinnata* as compared to the control treatment. Similar trends of growth and biomass production were obtained in all treatments after five months. Fertilizing *Azolla* with compost can be a good practice for the mass cultivation of *Azolla* but conclusive results are not available (Liu & Zheng 1992; Bonneto & Carcano 1995). Phosphorus is also essential for the optimal growth of *Azolla* (Watanabe *et al.* 1980; Aziz & Watanabe 1983; Watanabe & Ramirez 1990; Yanni 1991; Yanni *et al.* 1994). Best results can be obtained if phosphorus is supplemented with some nitrogenous fertilizer because synergistic effects of amelioration promote multiplication and enhance growth of *Azolla* (Yanni 1991; Yanni *et al.* 1994; Bonneto & Carcano 1995).

Table 1. Effect of various fertilizers on the population and biomass production in *Azolla pinnata*.

Treatment	May			August		
	No. of Plants	Fresh Wt. (g)	Dry Wt. (g)	No. of Plants	Fresh Wt. (g)	Dry Wt. (g)
Control	6066a	294.3a	17.9a	6233a	301.6a	15.5a
FYM	9293d	468.0d	25.5c	14800d	660.3d	30.7d
SSP	7000b	337.0b	20.1b	7300b	383.6b	19.6b
SSP + Urea	7830c	390.0c	23.3b	8000c	405.0c	25.3c

Values in each column followed by the same letters are not significantly different at $P \leq 0.05$.
FYM stands for farmyard manure and SSP for single super phosphate.

Results of this experiment suggest that farmyard manure is more effective and cheaper than phosphorus, and phosphorus plus nitrogen for multiplication and

biomass production of *Azolla pinnata*. *Azolla* may be a promising crop for vast areas of rice growing regions and it can also be a good substitute for substantial amounts of expensive nitrogen without environmental damage. *Azolla* can either be repeatedly harvested and incorporated as green manure or intercropped with rice to meet the fertilizer requirements of rice. However, further experiments are required to identify the stress factors affecting growth and multiplication of *Azolla*. Appropriate techniques which are agronomically feasible and socially acceptable also need to be developed for its economical propagation and use in rice. Future studies will be directed along these lines to work out solutions to these problems for sustainable farming systems.

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